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Experimental Studies on Toxicity of Ethylene Glycol Alkyl Ethers in Japan

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Past studies on the toxicological effects of ethylene glycol alkyl ethers as well as the recent data on these chemicals in Japan are reviewed. Only a few researchers have participated in the study of ethylene glycol alkyl ethers in Japan. The effects of ethylene glycol alkyl ethers on testis and embryotoxic effects of ethylene glycol monomethyl ether (EGM) have been studied, as has the teratogenicity of ethylene glycol dimethyl ether (EGdM). Studies on ethylene glycol alkyl ethers and related compounds administered to mice by oral gavage revealed the occurrence of testicular atrophy and decreased white blood cell count by EGM, EGdM, ethylene glycol monomethyl ether acetate, ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate, and the toxicity was related to their chemical structure. On the other hand, ethylene glycol, ethylene glycol monopropyl ether, ethylene glycol monobutyl ether, ethylene glycol monophenyl ether, ethylene glycol monoacetate or ethylene glycol diacetate showed no such an effect. Studies on EGM using hamsters or guinea pigs revealed the occurrence of testicular atrophy similar to that observed in mice. In regard to the methyl ethers of other glycols, there is no convincing evidence that propylene glycol monomethyl ether, diethylene glycol monomethyl ether or diethylene glycol dimethyl ether causes testicular atrophy in mice. Teratological studies of EGM and EGdM revealed embryotoxic effects in mice.

Introduction

In Japan, ethylene glycol ethers have been widely used as industrial solvents for resins, lacquers and dyes, but there are no available data on their use. The production of ethylene glycol ethers in Japan in 1980 is estimated to be 43,048 tons (*1*). The total amount of the ethers imported in 1980 was reported to be 8,121 tons, 94.5% of which came from the U.S. (*2*). Only a few researchers have participated in studies on the toxicity of ethylene glycol ethers in Japan. Since 1976, we have studied the effects of ethylene glycol alkyl ethers on the testis to define the possible relation between their chemical structures and the effects on the testis, and to elucidate the pathogenesis of testicular lesions (*3*). In addition, Uemura reported teratogenic effects of ethylene glycol dimethyl ether in mice in 1980 (*4*); we also reported embryotoxic effects of ethylene glycol monomethyl ether in mice in 1981 (*5*). Past studies on the toxicological effects of ethylene glycol alkyl ethers as well as recent data on these chemicals in Japan are reviewed in this paper.

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Testicular Atrophy Induced by Ethylene Glycol Alkyl Ethers

Three experiments were conducted to evaluate the toxicity of ethylene glycol alkyl ethers to the testis: (1) Testicular changes in mice given ethylene glycol alkyl ethers and related compounds; (2) the effects of ethylene glycol monomethyl ether on the testis of hamsters and guinea pigs; (3) The effects of alkyl ethers of other glycols on the testis of mice.

We attempted to determine the possible relation between their chemical structures and the effects on the testis, and the pathogenesis of testicular lesions in these experiments.

Testicular Changes in Mice Given Ethylene Glycol Alkyl Ethers and Related Compounds

Materials and Methods. Male JCL-ICR mice (Japan CLEA Co.), 6 weeks of age, were used. The materials

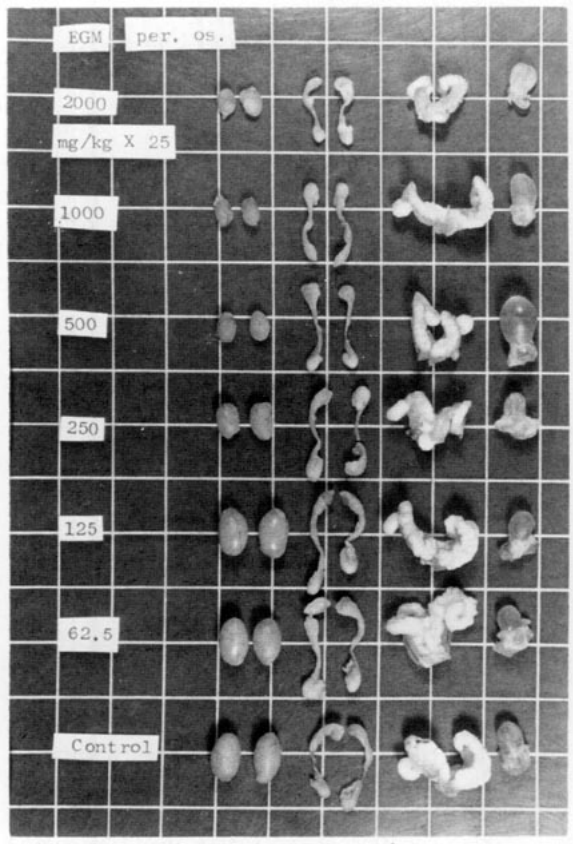


FIGURE 1. Testicular atrophy induced by EGM. Dose-dependent decrease in testis size is shown.

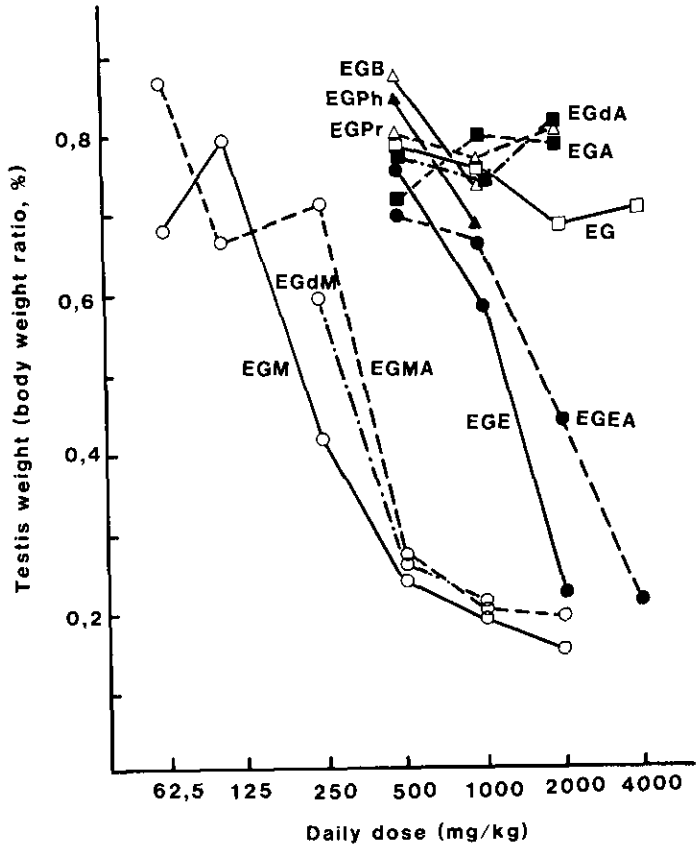
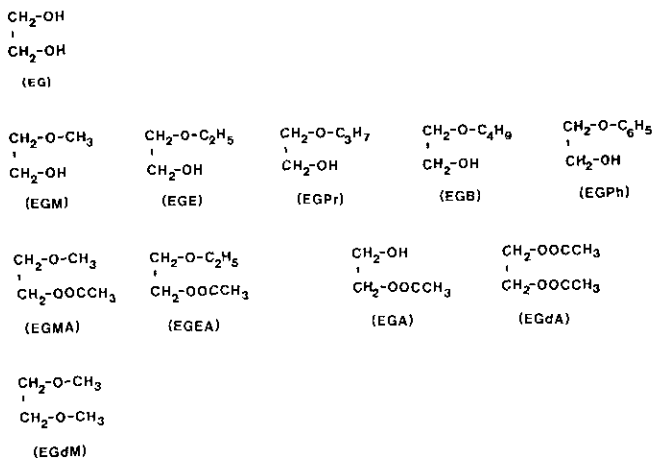


FIGURE 2. Relation between daily doses (expressed as mg/kg body weight) and testis weight of mice which were treated with ethylene glycol alkyl ethers and related compounds: (□——□) EG; (○——○) EGM; (●——●) EGE; (Δ---Δ) EGPr; (Δ——Δ) EGB; (▲——▲) EGPh; (○---○) EGMA; (●---●) EGEA; (■---■) EGA; (■—·—■) EGdA; (○—·—○) EGdM. Testis body weight ratio are expressed as the mean of the values from five mice at each dose.

tested were ethylene glycol (EG), ethylene glycol monomethyl ether (EGM), ethylene glycol monoethyl ether (EGE), ethylene glycol monopropyl ether (EGPr), ethylene glycol monobutyl ether (EGB), ethylene glycol monophenyl ether (EGPh), ethylene glycol monomethyl ether acetate (EGMA), ethylene glycol monoethyl ether acetate (EGEA), ethylene glycol monoacetate (EGA), ethylene glycol diacetate (EGdA) and ethylene glycol dimethyl ether (EGdM). EGPr and EGdA were obtained from Tokyo Kasei Kogyo Co., EGA from Kanto Chemical Co. and the rest from Wako Chemical Co.



Gastric intubation was employed for administration of each material because of the necessity of administering accurate amount of materials. Each of the samples was diluted with water or olive oil and given orally by stomach tube 5 days per week for 5 weeks. The daily doses were 4000, 2000, 1000 and 500 mg/kg body weight for EG, EGE and EGEA; 2000, 1000, 500, 250, 125 and 62.5 mg/kg body weight for EGM and EGMA; 2000, 1000 and 500 mg/kg body weight for EGPr, EGB, EGA and EGdA; 1000, 500 and 250 mg/kg body weight for EGdM. The control mice were given water by the same method. On the following day of the final administration, animals were necropsied under pentobarbital sodium anesthesia. The testes were weighed and the combined weight of seminal vesicles and coagulating gland was also measured. Tissue specimens for histopathological examination were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Blood for hematological studies was taken from the posterior vena cava at the time of necropsy.

Results. Gross observation showed that the testes of mice given some of the ethylene glycol alkyl ethers

were decreased in size (Fig. 1). The weight of the testes (expressed as testis–body weight ratio) of mice administered ethylene glycol alkyl ethers or related compounds for 5 weeks is shown in Figure 2. In animals given EGM, EGE, EGMA, EGEA and EGdM, dose-dependent decreases in testicular weight were observed. Considering toxic effects on the testis based on testicular weight and daily dose, as weight per body weight, EGM seemed to be most toxic, followed by EGdM, EGMA, EGE and EGEA, in that order. Esterification appeared to lessen the testicular atrophy effect in both the methyl and ethyl ethers of ethylene glycol, and dimethyl ethers had such toxicity less than monomethyl ethers. Examination of the testicular weight and daily molar dose per body weight (Fig. 3) showed that testicular atrophy was greater for EGM, EGMA and EGdM than for EGE and EGMA. The reduction in testicular toxicity by esterification or dimethylation when daily dose was expressed as weight per body weight was due to the difference in molecular weight of these compounds.

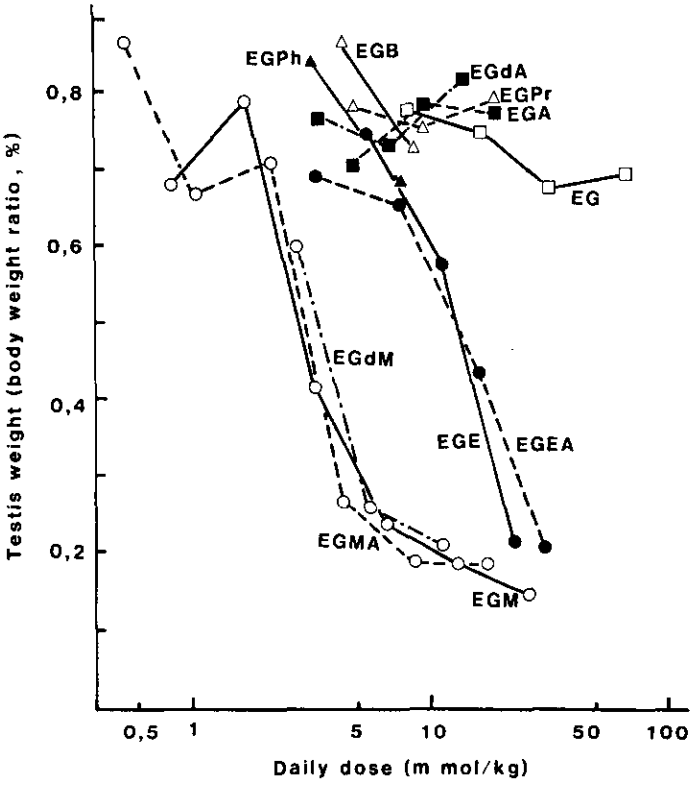


FIGURE 3. Relation between daily doses (mmole/kg body weight) and testis weight of mice which were treated with ethylene glycol alkyl ethers and related compounds. Symbols as in Fig. 2.

No significant difference in weight of the testis was found in EG, EGPr, EGB, EGPh, EGA or EGdA groups as compared with that of control animals. A slight decrease in the combined weight of the seminal vesicles and the coagulating gland was seen only in groups receiving higher doses of EGM, EGMA and EGdM.

Histopathological examinations of the testis from mice given EGM, EGE, EGMA, EGEA and EGdM revealed dose-related atrophy of the seminiferous epithelium at various degrees. Sertoli cells remained normal up to later stage. Leydig cells were normal in appearance, though infrequently proliferative (Fig. 4–13).

In the hematological examination, the decrease in white blood cell was the most prominent change. EGM, EGE, EGMA, EGEA and EGdM caused a significant decrease in white blood cell count, and the order of toxic

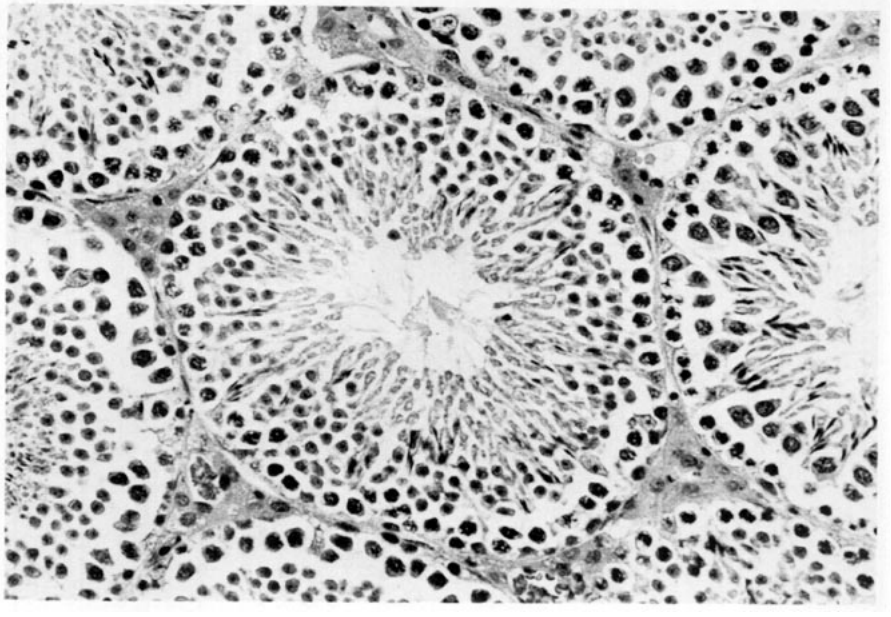


FIGURE 4. Testis of control mouse. H&E.

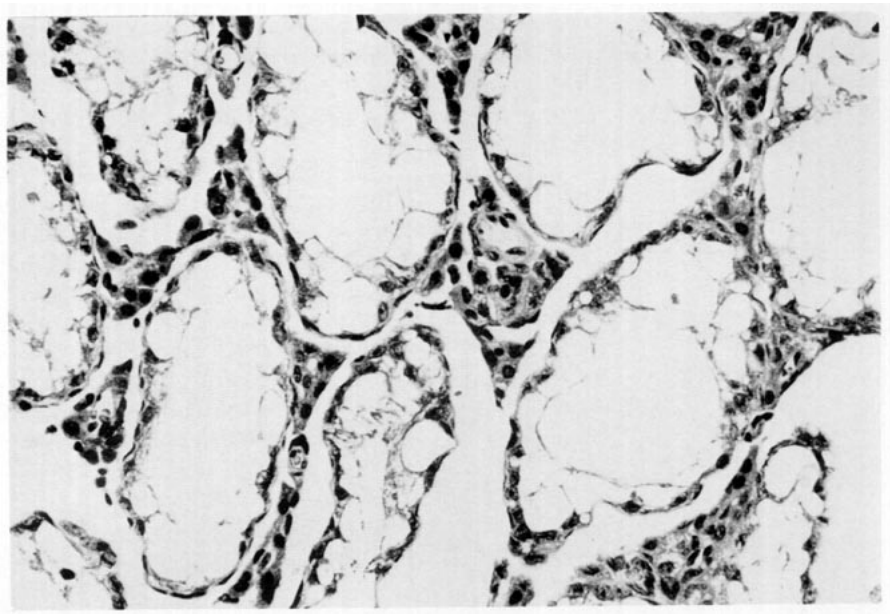


FIGURE 5. Testis of mouse treated with 2000 mg/kg/day of EGM. Seminiferous tubules are decreased in size and irregularly shaped. Spermiogenic cells have disappeared completely. H&E.

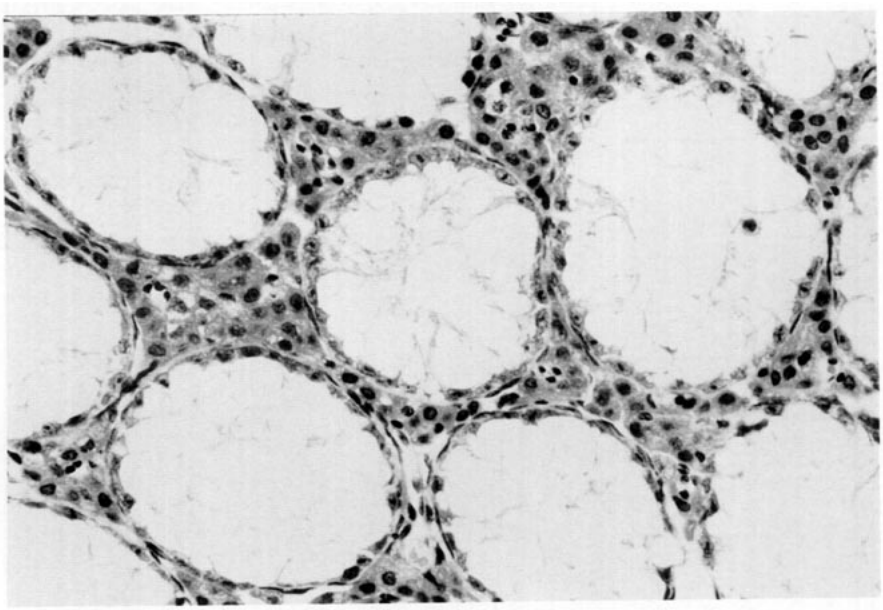


FIGURE 6. Testis of mouse treated with 1000 mg/kg/day of EGM. Small tubules with disappearance of spermiogenic cell cells are shown. H&E.

effect on leukocyte by these chemicals resembled that on testicular weight (Fig. 14). The change in red blood cells was slighter than leukocyte changes, and red cell count, packed cell volume and/or hemoglobin content decreased at the higher dose levels of EGM, EGMA, EGEA and EGdM. For EGB-treated mice, a decrease in red cell count was noted in 500 mg/kg or higher dose groups.

Effects of Ethylene Glycol Monomethyl Ether on Testis of Hamsters and Guinea Pigs

Since the effect of ethylene glycol alkyl ethers on the testis of other animal species was not known, we carried out preliminary studies of the toxic effect of EGM on the testis of hamsters and guinea pigs.

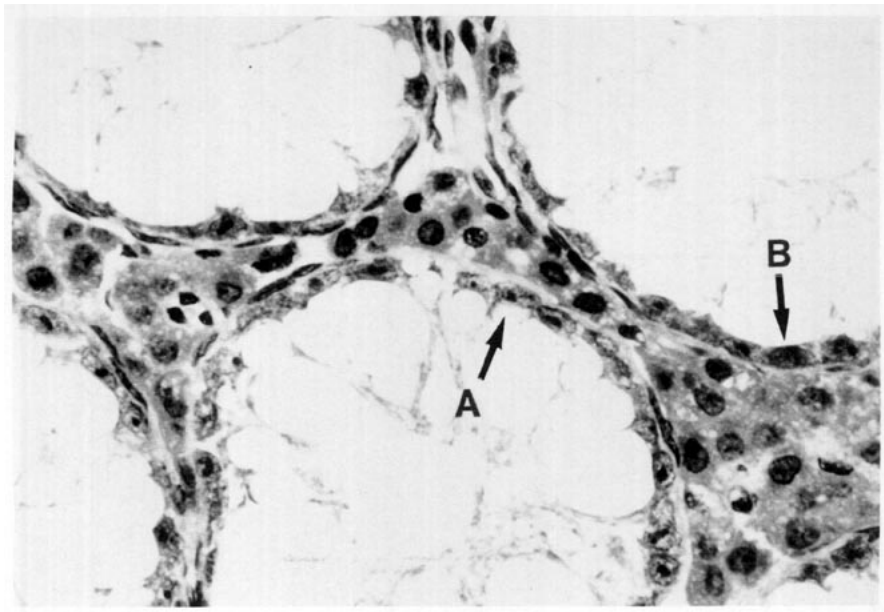


FIGURE 7. Same testis as in Fig. 6. Sertoli cells (A) and spermatogonia (B) are shown in the basal layer. H&E.

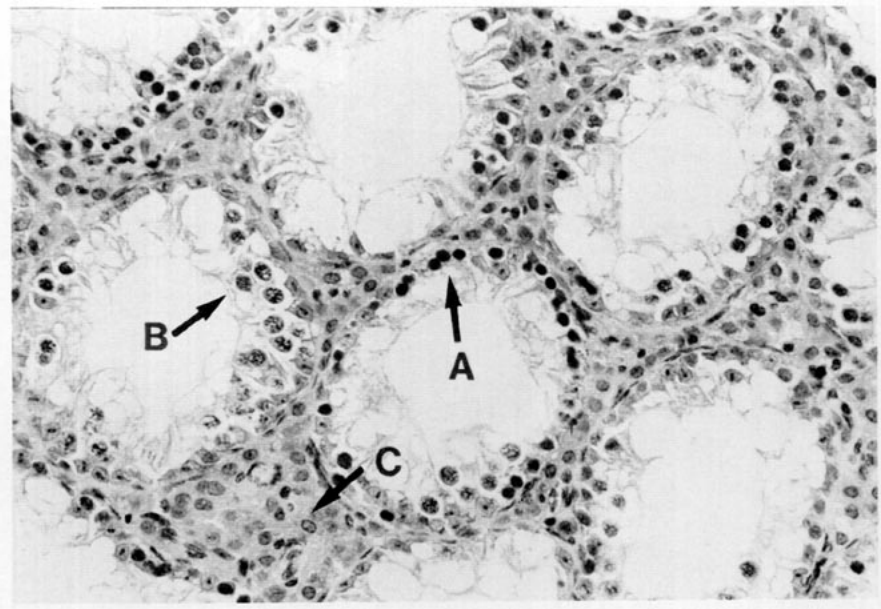


FIGURE 8. Testis of mouse treated with 500 mg/kg/day of EGM. Some spermatogonia (A) and spermatocytes (B) are preserved. Leydig cells (C) are proliferative. H&E.

Materials and Methods. Adult male Syrian golden hamsters and guinea pigs were used. EGM was diluted with water, and given orally with stomach tube 5 days per week for 5 weeks. The daily doses were 500, 250, 125 and 62.5 mg/kg body weight for hamsters, and 500 and 250 mg/kg for guinea pigs. The control animals were given water by the same method. On the following day of the final administration, animals were necropsied under pentobarbital sodium anesthesia. The testes were weighed and the combined weight of the seminal vesicles and coagulating gland was also measured. Blood for white blood cell count was taken from the posterior vena cava at the time of necropsy.

Results. A decrease in testicular weight was seen in all dose groups of hamsters (500, 250, 125 and 62.5 mg/kg body weight group), while neither the combined weight of the seminal vesicles and coagulating gland nor white blood cell count was affected (Table 1).

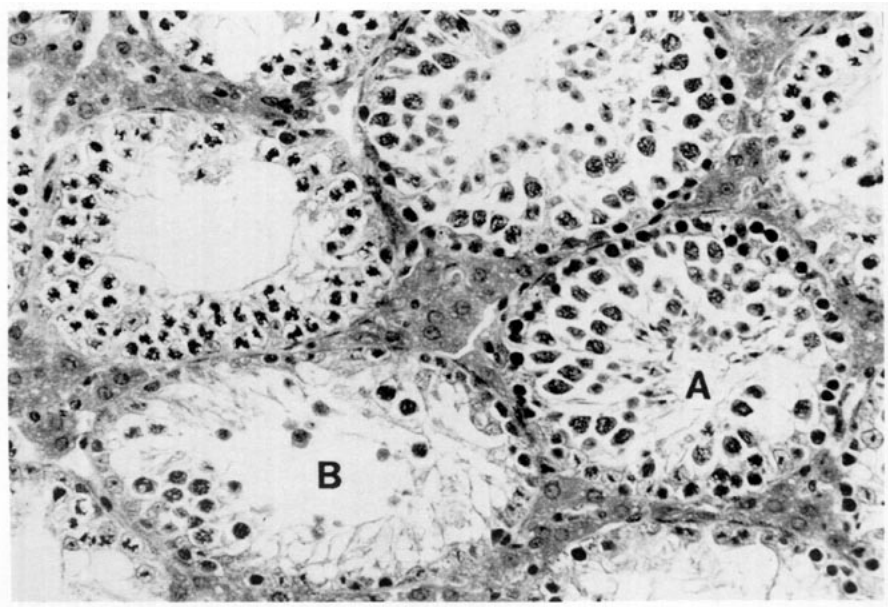


FIGURE 9. Testis of mouse treated with 250 mg/kg/day of EGM. Some tubules contain spermatozoa (A), but other tubules are atrophic (B). H&E.

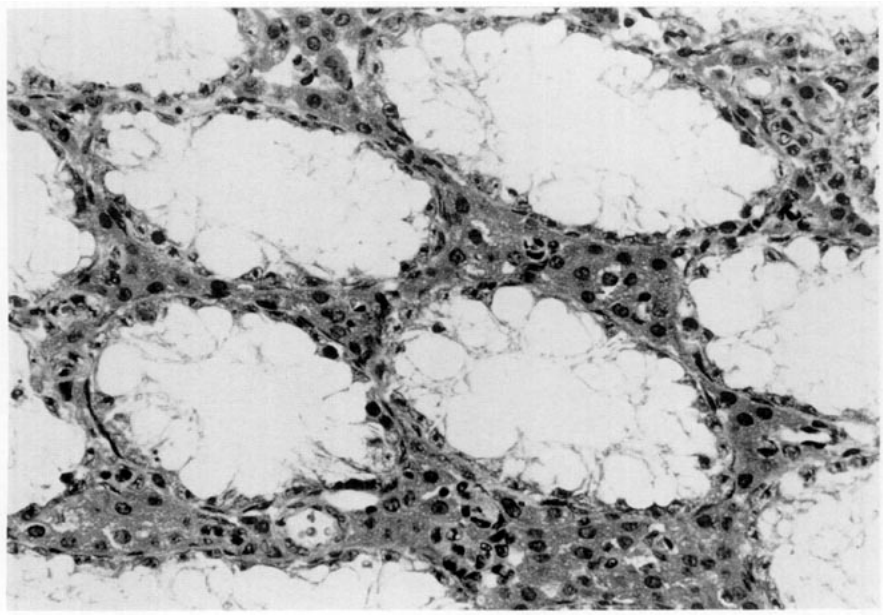


FIGURE 10. Testis of mouse treated with 1000 mg/kg/day of EGMA. Sertoli cells and a few spermatogonia are shown. H&E.

Table 1. Effects of ethylene glycol monomethyl ether on hamsters.

Daily dose, mg/kg	No. animals/ group	Mean body weight, g	Mean testis weight		Mean seminal vesicle and coagulating gland weight		Mean WBC/ mm^3
			mg	%[a]	mg	%[a]	
500	4	112	652[†]	0.58[†]	1172	1.03	4000
250	4	121	748[†]	0.62[†]	1543	1.29	4762
125	4	124	1299[†]	1.06[†]	1643	1.33	4675
62.5	4	131	3559	2.63[*]	1795	1.38	5433
Control	4	131	3715	2.84	1769	1.36	4925

[a]Organ/body weight ratio.
[*]Significantly different ($p < 0.05$) from the control group.
[†]Significantly different ($p < 0.01$) from the control group.

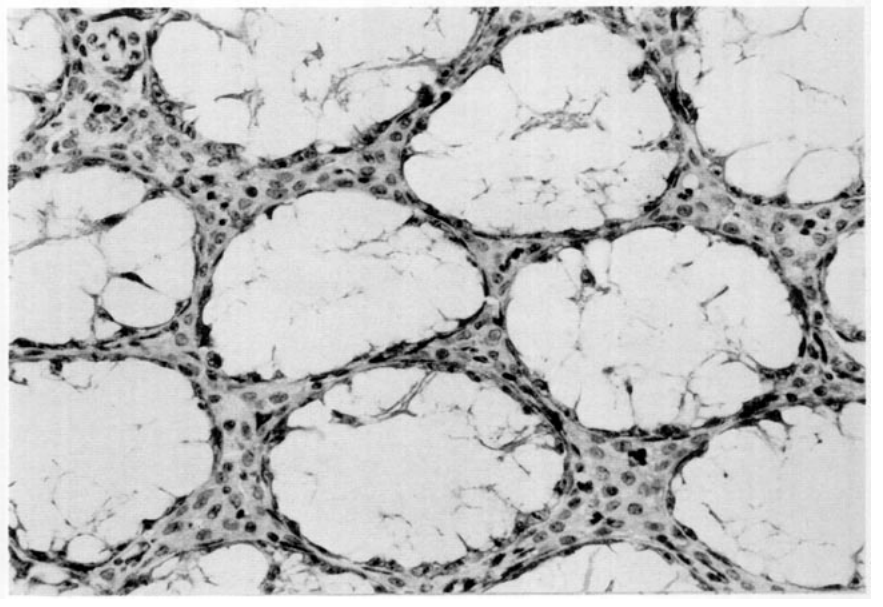


FIGURE 11. Testis of mouse treated with 1000 mg/kg/day of EGdM. Spermiogenic cells have disappeared. H&E.

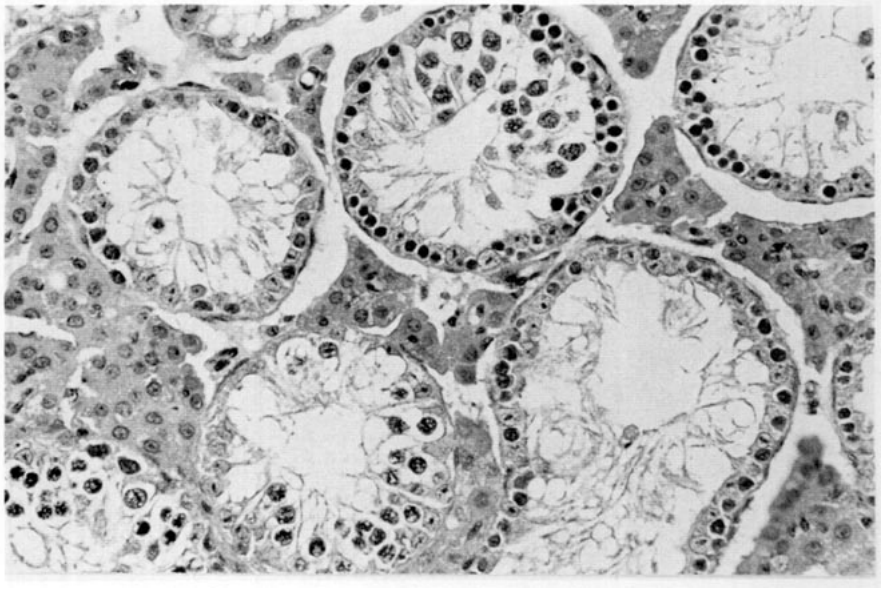


FIGURE 12. Testis of mouse treated with 2000 mg/kg/day of EGE. Some spermiogenic cells are preserved. H&E.

Table 2. Effects of ethylene glycol monomethyl ether on guinea pigs.[a]

Daily dose, mg/kg	No. animals/ group	Mean body weight, g	Mean testis weight		Mean seminal vesicle and coagulating gland weight		Mean WBC/ mm^3
			mg	%[a]	mg	%[b]	
500	3	782	927	0.12	4109	0.525	4000
250	3	755	946	0.13	3635	0.481	4100
Control	3	749	3889	0.52	3883	0.518	9180

[a]Statistical examination was not carried out.
[b]Organ/body weight ratio.

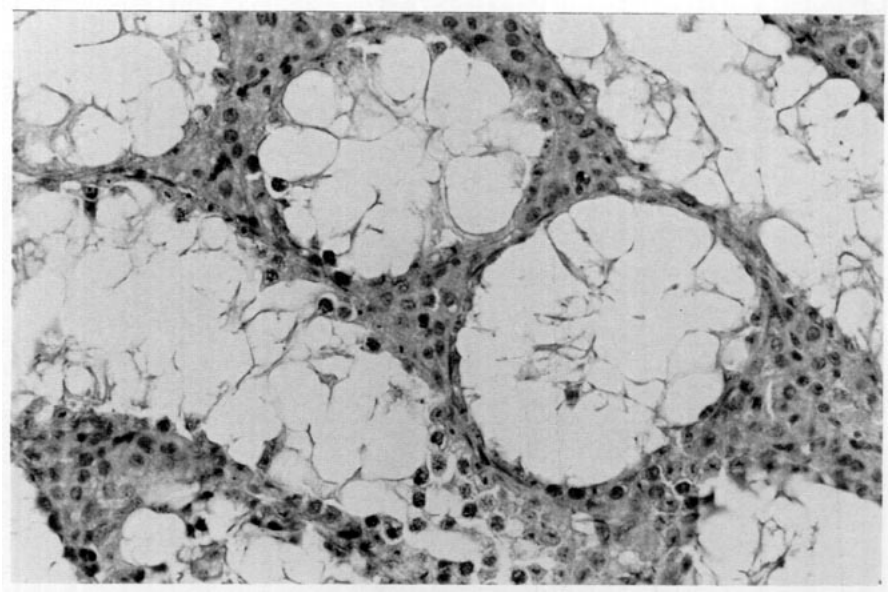


FIGURE 13. Testis of mouse treated with 4000 mg/kg/day of EGMA. Sertoli cells and some spermatogonia are preserved. H&E.

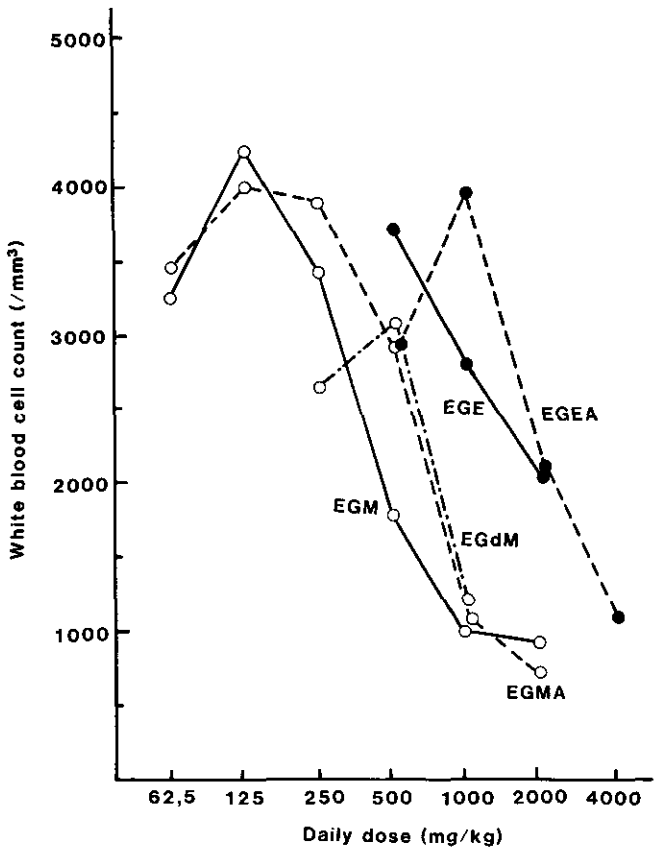


FIGURE 14. Relation between daily dose (expressed as mg/kg body weight) and white blood cell count of mice which were treated with ethylene glycol alkyl ethers and related compounds. (○——○); EGM; (●——●) EGE; (○---○) EGMA; (●---●) EGEA; (○——○) EGdM. White blood cell count is expressed as the mean of the values from five mice at each daily dose.

The testicular weight in all dose groups of guinea pigs (500 and 250 mg/kg) decreased to one-fourth of that in the control group, while the combined weight of seminal vesicles and coagulating gland was not affected. White blood cell count in all dose groups was decreased to one-half of that in the control group (Table 2).

From these results, it appears that oral administration of EGM causes testicular atrophy in hamsters and guinea pigs in the same manner as observed in mice.

Effects of Alkyl Ethers of Other Glycols on the Testis of Mice

Some alkyl ethers of ethylene glycols induce testicular atrophy in some animal species. In this experiment, we describe the possible effect of the methyl ether of propylene and diethylene glycol on the testis.

Materials and Methods. Male JCL-ICR mice (Japan CLEA Co.), 6 weeks of age, were used. The materials used were propylene glycol monomethyl ether (PGM), diethylene glycol monomethyl ether (dEGM) and diethylene glycol dimethyl ether (dEGdM), and EGM and EGE, as positive control. PGM was obtained from Tokyo Kasei Kogyo Co., and other chemicals from Wako Chemical Co. PGM, dEGM and dEGdM were administered to mice for 25 days in the drinking water at a level of 2% and EGM and EGE for 18 days at a level of 2.5%. Control mice were allowed water *ad libitum*. Animals were necropsied under pentobarbital anesthesia at the end of administration. Testicular weight and the combined weight of seminal vesicles and coagulating gland were measured. Blood for white blood cell count was taken from the posterior vena cava at the time of necropsy.

Table 3. Effects of alkyl ethers of other glycols on mice.

Compound	Concentration, %[a]	Duration of dosing, days	No. animals/group	Mean body weight, g	Mean testis weight		Mean seminal vesicle and coagulating gland weight		Mean WBC/mm^3
					mg	%[b]	mg	%[b]	
PGM	2.0	25	5	41.2	322	0.78	397	0.97	4530
dEGM	2.0	25	5	38.6	237	0.62	420	1.09	4690
dEGdM	2.0	25	4	41.0	218	0.53	381	0.93	3934
Control	0	25	5	40.6	290	0.71	441	1.09	3270
EGM	2.5	18	8	26.2†	47†	0.18†	191†	0.72	538†
EGE	2.5	18	8	33.7*	122†	0.36†	260	0.77	1506
Control	0	18	4	39.0	221	0.57	242	0.62	1740

[a]% in drinking water.
[b]Organ/body weight ratio.
*Significantly different ($p < 0.05$) from the control group.
†Significantly different ($p < 0.01$) from the control group.

Results. No significant difference was noted between PGM-, dEGM- or dEGdM-treated groups and control (in the testicular weight, the combined weight of seminal vesicles and coagulating gland or white blood cell count). In dEGM and dEGdM groups, a slight decrease in testicular weight was observed, but these decreases were due to one animal of each group and are statistically not significant. However, a decrease in testicular weight and/or white blood cell count was observed in both EGM-and EGE-treated groups. Under these experimental conditions, we can not indicate that PGM, dEGM or dEGdM causes any testicular atrophy (Table 3).

Embryotoxic Effects of Ethylene Glycol Monomethyl Ether and Ethylene Glycol Dimethyl Ether in Mice

It is well known that alkylating agents such as Busulphan inhibit cell proliferation and cause testicular atrophy and leukopenia. These chemicals produce a lethal effect or damage to the fetuses (*6*). The effects of ethylene glycol alkyl ethers on testis and leukocyte resembled those of alkylating agents. Consequently, ethylene glycol alkyl ethers which cause testicular atrophy and leukopenia may have also embryotoxic effects.

We studied the embryotoxic effects of EGM, and Uemura studied those of EGdM. These studies are summarized here.

Materials and Methods

Ethylene Glycol Monomethyl Ether (EGM). Female JCL-ICR mice (Japan CLEA Co.) were housed overnight with males of the same stock, and the day on which a vaginal plug was observed was designated day 0 of gestation. On days 7 to 14 of gestation, EGM at various daily doses (1000, 500, 250, 125, 62.5 and 31.25 mg/kg body weight) dissolved in water was given by gastric intubation. The group administered vehicle only served as the control. On day 18 of gestation, mice were sacrificed. Litter values and gross abnormalities were recorded. Blood was taken from dams for white blood cell count. Living fetuses with no external malformations or with only abnormal digits were examined for skeletal abnormalities and the degree of ossification.

Ethylene Glycol Dimethyl Ether (EGdM). Female CRJ:CD-1 mice (Charles River Japan Inc., 12 weeks of age) were housed overnight with same stock males (14 weeks of age), and the day on which a vaginal plug was observed was designated day 0 of gestation. On days 7 to 10 of gestation, EGdM at various daily doses (490, 350 and 250 mg/kg body weight) dissolved in water was given by gastric intutabion. The group administered vehicle only served as the control. On day 18 of gestation, mice were sacrificed. Litter values and gross abnormalities were examined. Fetuses were examined for their skeletal abnormalities and the degree of ossification.

Results

EGM. All fetuses except one of the 500 mg/kg group were dead in the 1000 and 500 mg/kg group, and there was an increase in the incidence of dead fetuses in the 250 mg/kg group. Gross abnormalities, such as

Although the significant maternal toxicity was not observed, both EGM and EGdM induced marked and dose-related embryotoxic effects. A summary of the results is given in Figure 15.

exencephaly, umbilical hernia or abnormal digits, were observed in the 250 mg/kg group. Reduction in fetal weight was seen in the 125 mg/kg and higher dose groups. Skeletal malformations including fusion and agenesis of either ribs or vertebrae were observed in 62.5 mg/kg or higher dose groups. Skeletal variations such as bifurcated or split cervical vertebrae and retardation of skeletal ossification in fetuses were noted in all treated groups (250–31.25 mg/kg).

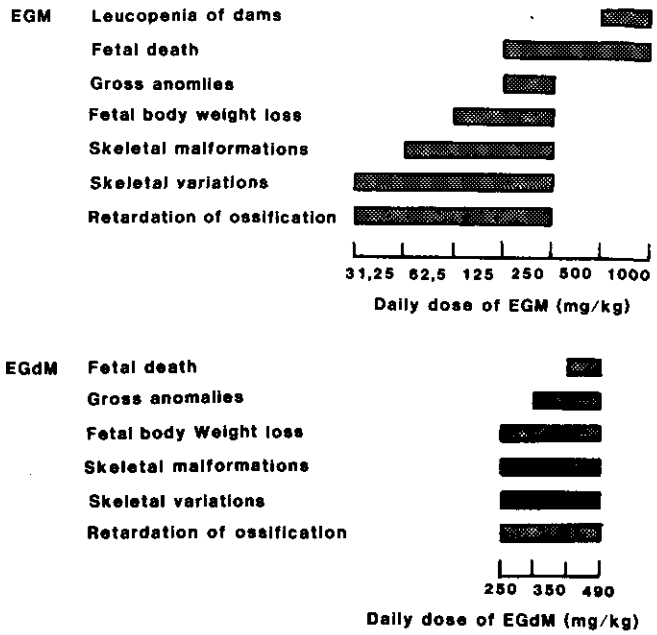


FIGURE 15. Summary of study on embryotoxic effects of EGM and EGdM.

EGdM. There was an increase in the incidence of dead fetuses in the 490 mg/kg group. An increase in number of gross abnormalities, such as exencephaly, caudal defect and umbilical hernia, were observed in the 350 mg/kg and higher dose groups. In all treated groups, increased incidence of skeletal malformations such as fused ribs and vertebrae, skeletal variations such as extra ribs and retardation of skeletal ossification was noted.

Discussion

Past studies and recent data on the toxicological effects of ethylene glycol ethers in Japan were reviewed. Testicular atrophy in mice, hamsters and guinea pigs were observed on administration of some ethylene glycol alkyl ethers, i.e., EGM, EGE, EGMA, EGEA and EGdM. A correlation between chemical structures and their effects on testis was observed. Testicular atrophy was induced by EGM, EGE, EGMA, EGEA and EGdM, and the atrophy was greater for EGM, EGMA and EGdM than EGE and EGEA, when dose level is expressed as mole/body weight. However, no significant effect on the testis was seen in the groups treated with EG, EGPr, EGB, EGPh, EGA and EGdA. Alkyl ethers of other glycols, i.e., PGM, dEGM and dEGdM, did not show any significant evidence on the induction of testicular atrophy in mice.

From the above findings it can be concluded that there is a correlation between chemical structures of ethylene glycol alkyl ethers and their ability to induce testicular atrophy. Both methyl and ethyl ethers of ethylene glycols cause testicular atrophy. Methyl ethers have a greater effect than ethyl ethers. Esterification of these chemicals does not affect the potency of the toxicity. Dimethyl ethers of ethylene glycols have quantitatively the same testicular toxicity as monomethyl ethers. Alkyl ethers of other glycols such as propylene glycol and diethylene glycol have no significant effect on the testis.

Ethylene glycol alkyl ethers can be concluded to induce testicular atrophy by affecting dividing cells of some kinds and inhibiting cell proliferation from the following reasons. Alkylating agents induce leukopenia as well as testicular atrophy by affecting cell division (*7*). Similarly, EGM, EGE, EGMA, EGEA and EGdM cause leukopenia at the same degree as for testicular atrophy. However, histopathologically, Leydig cells and Sertoli cells were found to be normal even at higher dose levels. Only a slight decrease in weight was recorded for the seminal vesicle and the coagulating gland at extremely higher dose levels. By these findings, testicular changes induced by ethylene glycol alkyl ethers can be distinguished from those induced by hormonal substances (e.g., 17 3-estradiol) or vasoactive substances (e.g., cadmium chloride) (*8*). Substances showing inhibitory effect on cell proliferation are known to produce a lethal effect on or damage to the fetus when given to pregnant animals (*6*). EGM and EGdM exhibit marked embryotoxic effects. This fact also supports the conclusion that ethylene glycol alkyl ethers have an inhibitory effect on cell proliferation.

From the results of the present animal experiments, it can be concluded that some ethylene glycol alkyl ethers have toxic effects on the testis and fetus.

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